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CLAIMS

#1

The publication of my UK patent entitled 'A surgical-medical dressing for the treatment of body burns and for wound healing' on February 13th, 2002 will arouse academic and commercial interest in HUVECs CM as well as my submitted 2001 paper.

If this dressing is to be used to treat a burns patient, it is envisaged that the whole dressing, will be enclosed in a gel-like substance, to facilitate easy handling by the attending physician. This gel-like substance will dissolve soon after being applied, i.e. contact with the patient's skin.

If this dressing is to be used in the treatment of a wound, it will be placed in the cavity of the wound and not removed. It is envisaged that several 'strips' of this dressing would be used depending on the size and depth of the wound.

It is envisaged that allogenic neonatal or infant keratinocytes (sparsely seeded) will be grown on the upper/outer layer of the dressing, if required for an 'occlusive dressing' use instead of an allograft/autograft.

Claim #2

**Human vein endothelial cells (HUVECs) conditioned
medium (CM) -----HUVECs CM.**

**The first scientific report of the tissue culture of
HUVECs was made by Jaffe et al. in 1973. This report
described the perfusion of an umbilical cord with an
enzyme, collagenase and the growth to confluence of
HUVECs. Basic fibroblast growth factor (b-FGF) or
commercially available endothelial cell growth
supplements (ECGS) are added to the nutrient solution,
(called the medium) to ensure that confluency is
attained. There are scientific reports by Gospodarowicz
et al. 1980a,b proving that assertion.**

Little attention has been paid to the conditioned medium

(CM) which is obtained after the nutrient medium is left

in contact with the proliferating cells for periods of time

which may vary from 2-3 days or longer. When the

medium is changed, the conditioned medium is always

discarded and an aliquot of fresh medium added.

In 1992, I published the first paper that showed a linkage

between the extracellular secretions of HUVECs and

human aortic endothelial cells. HUVECs were grown to

post confluence (2 days after normal confluence) and

the cell layer was detached with 5mM EDTA (ethylene

diamine tetra-acetic acid) leaving behind the sub-

endothelial matrix (the so-called extracellular matrix

(ECM)) intact. (The detached cells were reseeded onto a

fresh substrate and re-grown time and again.)

Human aortic endothelial cells were obtained by

mechanical scraping the intimal lining of a segment of

human aorta, and these endothelial cells were grown to

confluence on the HUVECs ECM, obtained as described

above. A postulate was proposed that ECMs had a

common phenotype.

In 2002, in a submitted paper to 'The International

Journal of Experimental Pathology', I illustrated the fact

that HUVECs CM may be used as a culture medium for

human dermal microvascular endothelial cells

(HDMECs), dermal fibroblasts, and epidermal

keratinocytes, because of related extracellular secretions (see Table I above). The CM may be pre-screened by bacteriological and other tests before use as required.

DISPASE, a neutral protease is used to detach epithelial sheets (epidermal keratinocytes are grown in sheets in a tissue culture laboratory) which are used clinically as cultured epithelial autografts (CEAs) in the management of body burns.

Because CEAs are manufactured using Dispase, the 'take' of the graft is impaired (Rennekampff et al. 1996).

The scientific paper describing Dispase was published in 1992. The title of the paper was: Dispase, a neutral

protease from *Bacillus polymxa*, is a powerful

fibronectinase and Type IV collagenase.

HUVECs secrete into their conditioned medium (CM),

Type IV procollagen, fibronectin and thrombospondin

which makes the HUVECs CM a natural neutralizing

agent for Dispase.

Cascade Biologics, Inc. (Portland, OR), to name just one

company in this line of business, sell all types of tissue

culture media, cell types, ECGS etc. but not HUVECs

CM. Interestingly, they sell pooled HUVECs, from more

than one umbilical cord. The resultant ECM will not be

pure as an original from one umbilical cord.

Scientific references:

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Rennekampff H.O., Kiessig V. & Hansbrough J.F. (1996)

Research Review-Current concepts in the development

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Pathol. The inter-relationships between human

endothelial cells, dermal fibroblasts and epidermal

keratinocytes extracellular secretions, and their

connection to wound healing.

Claims

3

By-product

A can spray of cornstarch and oil of cloves mixture.

Presently, a product called 'Water.Jel' (Carlstadt, NJ) is used by City of Miami paramedics in onsite, first aid treatment of burn victims. My suggestion was initially an antibiotic-100% cornstarch spray in a durable container like a can spray. Enquiries have revealed that 'Water.Jel', a soothing wrap, suffers from fungi infection, and puncture of its packaging. Instead of using an antibiotic, and circumventing FDA approval, a mixture of oil of cloves and 100% cornstarch, packaged in a spray can is contemplated as a by-product.

Why cornstarch? It is a white granular carbohydrate occurring in the endosperm of corn kernel. It enjoys wide use as a major constituent of talcum body powder, in domestic cooking as a thickener, (a binding agent), for soups and stews and in the laundry product, spray starch. Cornstarch is used to soothe sunburn, for the relief of diaper rash, prickly heat, and itchy, irritated skin.

On arrival on hospital property, this white coating could be easily removed by salving the affected area. Alternatively, if maggots are to be used, they can be applied without salving. Anything that can stem tissue bleeding, loss of oozing wound fluid, and provide some small measure of pain relief, at the site of the incident, will be an improvement on 'Water.Jel'.

This proposed spray should compromise the blood coagulation cascade, and the release of cellular growth

factors e.g. PDGF and alpha-thrombin, which cause recruitment to the wound bed and both the proliferation and migration of fibroblasts.

It will also approximate an occlusive dressing, because there will be a vapor barrier. Minutes of the so-called 'golden hour' might be used beneficially, or even saved.